Role of leafing phenology in the invasion of forest ecosystems by *Rhamnus cathartica*

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Abstract

The introduction of invasive species is associated with the degradation of native habitats. Invasive species tend to form dense monocultures that crowd out native flora decreasing biodiversity, changing successional pathways, and degrading ecosystem services. How these species are able to thrive is not fully understood. This thesis focuses on the role of phenology in facilitating the colonization of the woody shrub *Rhamnus cathartica* (buckthorn) in northern deciduous forests.

Buckthorn breaks bud earlier in the spring and holds leaves later in the fall compared to co-occurring native understory species and the forest canopy. This phenology may allow buckthorn to take advantage of high light levels prior to canopy closure in spring and after leaf drop in fall. We hypothesized that this unique phenology is one mechanism that facilitates invasion of the forest interior by buckthorn. To test our hypothesis, we experimentally shaded buckthorn seedlings, reducing high light levels in the spring and fall to simulate intact canopy conditions. Forty individuals at two sites in central MN, USA were randomly assigned to four treatments: spring shading, fall shading, both spring and fall shading and no shading. We measured spring and fall leafing phenology, light availability and seedling survival and growth. After a year and half of shading little mortality was observed but individuals receiving shading treatments had significantly decreased growth. Supporting our hypothesis that access to

phenology-induced high light levels in the spring and autumn is one mechanism for buckthorn success in closed canopy forests.

To understand why buckthorn was breaking bud sooner than native vegetation we investigated the winter chilling requirements of buckthorn, as proper winter chilling is required for most temperate plants to break bud in the spring. Exposing buckthorn branches to increasing levels of winter chilling demonstrated that buckthorn has a low winter chilling thresholds, allowing it to respond faster to spring forcing temperatures than native species.

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Introduction

Light availability and species traits shape community assemblages in deciduous forest ecosystems (Lopez et al. 2008). In these communities the canopy can intercept over 98% of incoming light, making light availability a key limiting resource (Brown and Parker 1994). Many plants cannot survive at these low light levels, but understory plants have developed strategies to maximize light capture and lower physiological requirements, allowing them to thrive in low light (Valladares and Niinemets 2008).

Invasive species tend to be most prevalent in disturbed open areas where there are ample resources available for the invasive species to exploit. A lack of predators and high fecundity allow them to quickly spread and dominate the community (Lake and Leishman 2004). For some time, it was thought that high competition for light resources prevented invasive species from spreading into closed canopy forests (Martin et al. 2008). However, a number of non-native species have been identified as invasive in low-light forest understories (Martin et al. 2008), where they outcompete native vegetation, causing biodiversity loss (Hejda et al. 2009), changing ecosystem structure and function (Kourtev et al. 2002), and changing the successional pathways of the forest (Martin et al. 2008). Why these species are so successful is not fully understood.

One line of evidence suggest that invasive species use phenology to gain a competitive advantage (Fridley 2012). Growing season length for invasive species tends to be longer than that of co-occurring native species (Knight et al.

2007, Willis et al. 2010, Fridley 2012, Polgar et al. 2014). Some species, like *Alliaria petiolata (*garlic mustard*),* have evergreen leaves (Jean Engelhardt and Anderson 2011), whereas others exhibit early leaf out in the spring and later leaf senescence in the fall (Harrington et al. 1989, Xu et al. 2007, McEwan et al. 2009, Polgar et al. 2014). By breaking bud earlier in the year and holding onto leaves later, invasive species have longer growing seasons than native plants, which gives them access to more resources.

Early leafing and late leaf drop strategies also benefit from the seasonal nature of light in deciduous forests. Closed canopy forest light levels are typically less than 5%, but in the spring and fall when the canopy is leafless, greater than 50% of the above canopy light can reach the forest floor (Augspurger et al. 2005). Tree seedlings have been shown to take advantage of high light levels early in the growing season by breaking bud before overstory adults (Augspurger 2004, Lopez et al. 2008, Lu et al. 2012). For example, *Acer saccharum* (sugar maple) and *Aesculus glabra* (Ohio buckeye) seedlings leaf out two weeks earlier than the canopy trees of the same species.

There is evidence that invasive species may also take advantage of these high light levels. A study in New York with 30 invasive species found that estimates for annual carbon gain range from 1% to 29% in the spring and between 1% to 21% in the fall for invasive species (Fridley 2012). Early spring leaves of *Rhamnus cathartica* (buckthorn), *Lonicera mackii* (Amur honeysuckle), and *Berberis thunbergii* (Japanese barberry) are high in nitrogen and chlorophyll

(Harrington et al. 1989, Xu et al. 2007) allowing them to fix large amounts of carbon prior to spring canopy closure. Comparisons of non-native species with a co-occurring native species *Cornus racemosa* (gray dogwood) show that in the spring, buckthorn and Amur honeysuckle gained 35% and 29% of their annual carbon gain while gray dogwood was dormant. In the fall, 9% annual carbon gain for buckthorn and 12% annual carbon gain was accumulated, after gray dogwood had gone dormant for the season (Harrington et al. 1989). These large pools of spring and fall carbon that the invasive species were able to synthesize while co-occurring natives were dormant could provide a competitive advantage that may explain the success of these two species in their introduced range.

To date, only one study has quantified the role of high light levels prior to canopy closure on the growth and survival of invasive species in a closed canopy forest. The invasive forb garlic mustard was shaded from early April to canopy closure in mid-May to remove the high pulse of spring light to see how important it was for the growth and reproduction of this plant. Shading garlic mustard individuals prior to canopy closure in the spring resulted in a 50% reduction in shoot and root growth and a 75% reduction in fruit production (Jean Engelhardt and Anderson 2011).

While no invasive woody species have been tested, similar results have been seen in native tree species. *Acer saccharum* (sugar maple) and *Aesculus glabra* (Ohio buckeye) seedlings leaf out two weeks earlier than the canopy trees of the same species. In a three year study, shading seedlings early in the spring

to remove the early spring high light “window” led to a significant decrease in growth and survival of the two species (Augspurger 2008), suggesting that carbon gain from early leaf phenology confers shade tolerance to individuals and is necessary for survival in the deeply shaded summer months.

If phenological differences between seedling and canopy trees provide a competitive advantage, invasive species that tend to be the first species to break bud in spring could also have increased fitness because of their phenology. In the eastern and upper Midwest of the USA, buckthorn is a non-native woody invader that appears to be following this pattern. Previous studies have demonstrated that North American populations of buckthorn have a longer growing season than native shrubs (Harrington et al. 1989, Fridley 2012). Buckthorn breaks bud earlier in the spring and holds onto its leaves later in the fall, compared to other woody understory species and the forest canopy (Becker et al. 2012). In similar studies in Europe, buckthorn did not have a longer growing season than other co-occurring woody shrubs (Knight et al. 2007). These changes in phenology may be having an effect on the spread of buckthorn. In North America, buckthorn readily invades sites in the forest interior (Knight et al. 2007), whereas European populations grow largely on brighter forest margins (Godwin 1943, Knight et al. 2007).

Here I present a study that examined whether phenological differences between common buckthorn and canopy trees provide windows of high light in the spring and fall important for the growth and survival of common buckthorn in

the forested understory. I hypothesized that without access to high light levels in the spring and fall, buckthorn will show decreased growth and increased mortality. To test this hypothesis, buckthorn seedlings were shaded in the spring and the fall to remove the pulses of high light received while photosynthetically active and the canopy is dormant. I expected decreased growth and increased mortality in shaded seedlings compared to unshaded seedlings. Since levels of spring carbon gain have been found to be higher than those in the fall, I expected that the decrease in growth would be stronger for those shaded in the spring when compared to the individuals shaded in the fall. Plants shaded in both the spring and fall should exhibit the largest decreases in growth and survival.

Materials and Methods

Individuals and Study Sites

The study was conducted in two research forests in east central Minnesota: Cedar Creek Ecosystem Science Reserve (Cedar Creek), and Lee and Rose Warner Nature Center (Warner Nature Center). Cedar Creek is a 2,200 ha University of Minnesota research station located 55 km north of St. Paul, MN (45^{o}25'N, 93^{o}10'W), with a mean annual temperature of 6.7^{o}C and mean precipitation of 72.6 cm/year. Warner Nature Center is a 243 ha private nature center 48 km northeast of St. Paul, MN (45^{o}11'N, 92^{o}50'W), with a mean annual temperature of 7.0^{o}C and mean precipitation of 80.7 cm/year. The research site at Cedar Creek was located in deciduous forest dominated by *Quercus macrocarpa* with a seedling layer dominated by common buckthorn with

some *Zanthoxylum americanum*. The research site at Warner Nature Center was located in mixed upland deciduous forest with a canopy comprised of *Quercus macrocarpa* and *Acer rubrum* and a seedling layer dominated by common buckthorn. Newly invaded woodlands without a history of management were selected to avoid plants arising from stem resprouts or root sprouts associated with a larger plant.

In March 2012, 40 buckthorn seedlings less than 60 cm in height were haphazardly selected within a 0.5-ha research area at each site. No study individual was located within a 3-meter radius of another study individual. Since light levels can vary significantly on the edge of stands or near tree fall gaps, only individuals greater than 25 m from the forest edge and tree fall gaps were included. In May 2013, a second cohort of 40 plants at each site was added to the study. Each cohort was analyzed separately. To differentiate between the two years, plants selected in March 2012 will be referred to as cohort one and plants added in May 2013 will be referred to as cohort two.

Plants were randomly assigned to one of four treatment groups each containing ten seedlings: ambient, spring shaded, fall shaded, and both shaded. One individual in the spring shaded group was dropped from the study after discovery of a root graft with another individual. Plants in the spring shaded and both treatment groups received shading in the spring while the fall shaded and both treatment groups received shading in the autumn. The ambient treatment group received natural light for the duration of the study.

Shade treatments consisted of a single layer of 80 percent neutral density black shade cloth stretched over a 70 cm square 1-meter tall PVC frame. Installed in a north-south orientation, shade cloth was left open 20 cm from the ground on the east, west and south sides, and 50 cm from the ground on North side to allow for air circulation. In 2012, spring shading was applied from bud break to 95% canopy closure and in the fall when the canopy was 95% open until buckthorn leaf senescence. During the second year of treatment, a less aggressive shading treatment was used to prevent shaded individuals from receiving light levels below 1% percent transmittance. In 2013, spring shading was from bud break to 80% canopy closure and fall shading was from 80% canopy opening to buckthorn leaf senescence (Table 1).

Light

Percent transmittance was measured weekly during the treatment and approximately monthly during the summer using a paired sensor approach. Open readings of photosynthetic active radiation (PAR) were taken in a field 200 m from the Cedar Creek site and 400 m from the Warner Nature Center site to capture the total irradiance above the canopy. The sensor was placed 1.5 m above the ground in a horizontal position with an unobstructed view of the sky (>25 m from the nearest tree), and PAR readings were recorded every minute (Accupar LP-80, Decagon Devices). A hand-held quantum sensor positioned 5 cm above the top of the plant was used to measure PAR reaching each plant (LI-

250A, Li-COR Biosciences). PAR measured at each seedling was matched to the corresponding open measurement and the percent transmittance was calculated by dividing the PAR at the plant by the PAR at the open PAR and multiplying by 100. In 2012, all plants were measured. Based on 2012 results, I reduced the sample size in 2013, measuring 5 individuals chosen at random from each treatment at each site.

Phenology

The phenological state of each plant was monitored weekly from March to June, and once per month in July and August. Weekly readings were resumed in September until leaf fall in November. Four phenological states were monitored: opening of bud scales (bud break), leaves elongated and in correct orientation (expansion), break down of chlorophyll (senescence), and abscission of leaves (leaf fall). Plants were considered to have reached these states when 50% of their leaves reached the state.

Growth

Plant size measurements were obtained prior to bud break in the spring and at the end of the growing season in September when buckthorn branches had stopped elongating. Stem diameter was measured at the first knot-free section of the stem at least 5 cm above the ground. During the initial measurement, this spot was marked with an acrylic paint pen, and all subsequent

measurements were taken at the same spot. To account for the stem not being perfectly round, I made 5 readings approximately 35 degrees apart with micro calipers. I measured height to the terminal bud. To capture apical growth, the elongation of all branches during the growing season was recorded by measuring the distance from the previous years bud scar to the tip of the branch.

Insect damage

In May 2012 at shade structure removal, a higher frequency of insect herbivore activity was observed on the spring shaded and both shaded treatments. To quantify the differences among groups, I surveyed herbivore damage to each plant on May 22, 2012, by examining all leaves on each plant. Each leaf was classified as having no damage, less than 25% missing (low), 25% to 50% of the leaf missing (medium), and greater than 50% of the leaf missing, including new leaf scars (high). The percentage of leaves in each damage classification state was calculated by dividing the number of leaves in each category by the total number of leaves and multiplying by 100. The number of caterpillars on each plant was recorded and representative samples of all species were collected and identified in the lab. All caterpillars were manually excluded from the study at this time to ensure that the herbivore pressure was zero for all plants after the shade structures were removed.

Based upon the results of the herbivore survey in 2012 the following steps were taken to ensure that shade structures did not attract more caterpillars to shaded plants in the spring of 2013. The less aggressive shade treatment

removed the shade structures earlier in the spring, decreasing the chances that caterpillar emergence would coincide with shade treatments. All study plants were monitored weekly for the presence of caterpillars and herbivore damage during the spring 2013 shading period. Caterpillar activity was detected on May 16, 2013 two days prior to shade structure removal at Warner Nature Center. At this time, I repeated the herbivore damage survey that was completed in 2012 to characterize the level of damage. To prevent the shade structures from attracting more caterpillars during the remaining shading treatment, all treatments in both cohort one and cohort two at Cedar Creek and Warner Nature Center were sprayed with the insecticide *Bacillus thuringiensis kurstaki* on May 16, 2013 after the completion of the insect survey.

Statistical analysis

To evaluate the relationship between insect herbivory and shade treatment, two-way analysis of variance (ANOVA) was used. The model included the fixed effect of treatment and site as a random effect. Response variables included: percentage of leaves eaten, percentage of leaves with 25% or less insect damage, 25% to 50% of leaves with insect damage, leaves with greater than 50% of the leaf damaged or missing, and number of caterpillars present. To compare the differences in plant growth we performed two-way analysis of covariance (ANCOVA). The analysis included the fixed effect of shade treatment with initial stem diameter as a covariate and site as a random effect. To examine the effects of herbivore damage on growth, we used a mixed model with

response variable of change in stem diameter and main effects of herbivore damage and shade treatment. Site was a random effect. All statistical calculations were performed in JMP Pro 11 (Statistical Analysis Software).

Results

Light

The 2012 spring treatment at Warner Nature Center was applied on March 17, 2012 and reduced percent transmittance from 45% for ambient seedlings to 5% for shaded individuals. Percent transmittance remained constant until the canopy began to leaf out on April 13, 2012 at which point light levels of ambient plants began to decrease eventually reaching 5% on May 15, 2012 (Figure 1A). At this time the shade structures were removed. Cedar Creek underwent a similar pattern (Figure 1B). During the summer percent transmittance remained at 2% until September.

On September 14, 2012 at Warner Nature Center and September 16, 2012 at Cedar Creek, falling canopy leaves caused light transmittance values at the two sites to rise above 5% transmittance, and at this time shading was initiated for the fall treatments. At Warner Nature Center ambient light levels rose from 9% to 53% transmittance between September 14, 2012 and November 3, 2012 when the canopy lost all of its leaves. Light levels remained just above 50% transmittance until buckthorn lost all of its leaves and shade structures were removed on November 28, 2012 (Figure 1C). Cedar Creek light levels followed

the same pattern, with removal of shade structures occurring on the same date, November 28, 2012 (Figure 1D).

In 2013 a more conservative deployment of shade structures was enacted to prevent light levels from dipping below 1% transmittance for treated individuals. At Warner Nature Center, treatment began on May 1, 2013 and shade treatments were removed at 20% light transmittance, May 28, 2013. Fall shading was initiated at 20% light transmittance (October 29, 2013) and continued until buckthorn leaf fall (November 13, 2013) (Figure 1D). Light levels and shading times were similar for Cedar Creek (Figure 1C).

Phenology

Cohort one experienced no differences in the timing of bud break or senescence during the 2012 growing season (Table 2). Shade treatment was a significant predictor for leaf expansion ($F_{3,74} = 3.60$, $p = 0.02$). Ad hoc Tukey HSD tests revealed that the fall shaded group was significantly delayed when compared to the ambient group during leaf expansion, but at this point both the ambient group and the fall shaded group had received the same treatment. Therefore, differences in leaf fall time cannot be attributed to treatment. Plants shaded in spring and fall had significantly earlier leaf drop when compared to the other three groups ($F_{3,74} = 7.50$, $p = 0.0002$).

In 2013, treatment was a significant predictor of timing of leaf expansion ($F_{3,74} = 8.83$, $p < 0.0001$) of cohort one. Plants in the spring shaded and both

shaded groups had significantly delayed leaf expansion compared to the fall shaded and ambient groups (Tukey's HSD; $p<0.05$). The two treatments that underwent spring shading were six days delayed in reaching full leaf expansion when compared to the ambient and fall shaded groups in 2013. There were no differences for bud break, senescence, or leaf drop for cohort one. In cohort two plants undergoing spring shading were 4 days slower in reaching full leaf expansion. There were no differences for bud break, senescence, and leaf drop among groups (Table 3).

Insect Damage

Three caterpillar species from family Lepidoptera: tortricidae were identified feeding on buckthorn in May 2012. One specimen was identified to the species level as *Archips purpurana* (an omnivorous leaf rolling caterpillar), the other two were only identified to the family level. The number of caterpillars observed on each plant was quite low (0.5 $\pm$ 0.2 SE caterpillars), with no treatment effect observed.

Herbivore damage from caterpillar feeding was higher for the individuals that were shaded in the spring (Figure 2). Herbivore damage was observed on 42% of leaves on shaded plants compared to 9% for unshaded plants ($p< 0.0001$). Additionally, the magnitude of the damage was also higher for shaded plants. Leaves with more than 25% of their surface area were ten times more likely to be found on plants undergoing spring shading (Figure 2). We found no relationship between change in stem diameter and herbivore damage ($F_{1,78}$ =

0.816, p = 0.37, Figure 3). Figure 3 clearly shows that plants shaded in 2012 had no growth regardless of the amount of insect damage that was present. Given these results, insect damage was not included in models analyzing growth for this study.

Insecticide treatments applied in 2013 were very effective at controlling insect damage that was associated with shading structures in the prior year. I first observed caterpillar feeding two days prior to shade removal. One caterpillar was found on each of the ambient, spring shaded, and both shaded treatments. No caterpillars were found at Cedar Creek prior to shade structure removal. Herbivore damage was measured three days after shade structure removal and herbivore damage levels were less than 5% for all groups, with no significant differences for plants in cohort one (Figure 4). There was a small but statistically significant difference ($F_{1,78} = 5.64$, $p = 0.02$) in total herbivory between the ambient (3% $\pm$ 1% SE) and spring shaded (6% $\pm$ 1% SE) treatment groups in cohort two (Figure 4). When included in the growth measurement models this herbivore damage was not a significant predictor of growth, and it was dropped from the models.

Growth data

Noticeable changes in growth patterns were visible by the end of the first growing season, and continued into the second growing season for cohort one. Treatment was a significant predictor of change in stem diameter in 2012 ($F_{3,76} = 15.17$, $p < 0.0001$) and 2013 ($F_{3,76} = 15.68$, $p < 0.0001$). Tukey HSD tests

revealed that spring shading was responsible for this difference as the spring shaded and both shaded groups had significantly reduced changes in stem diameter when compared to the ambient and fall shaded groups in 2012. By 2013, spring shading, both shading and fall shading treatment groups all had significantly reduced stem diameter growth. Height growth did not differ among shade treatments in 2012 ($F_{3,76} = 1.34$, $p = 0.25$), but there were significant differences among treatments in 2013 ($F_{3,76} = 3.74$, $p = 0.01$, Figure 7). The difference in the model was caused by the ambient group being significantly taller than both the spring shaded and both shaded groups when analyzed with Tukey HSD Tests. Treatment was a significant model predictor of branch elongation in 2012 ($F_{3,76} = 3.07$, $p = 0.03$), with Tukey HSD tests assigning the significance differences to smaller average elongation in the both shaded group compared to the fall shaded group. In 2013, treatment was not a significant predictor of branch elongation ($F_{3,76} = 1.33$, $p = 0.27$).

Growth metrics for Cohort 2 in 2013 showed similar results to cohort 1 during their first season of treatment in 2012. Spring shaded plants had reduced stem diameter growth compared to ambient grown plants ($F_{3,75} = 11.5$, $p < 0.0001$, Figure 8). Height was unaffected by 2013 shading in cohort 2 ($F_{3,75} = 1.9$, $p = 0.14$)

Mortality

All plants survived through the first growing season. Two cohort one individuals were dead after the second growing season: one in the both shaded

and one in the spring shaded groups. There was no mortality in cohort two. Sample size of dead shrubs was not large enough to analyze statistically.

Discussion

Buckthorn is normally found on forest margins in its native range but is able to colonize the forest interior in North America. This study documents the role of early leaf out in the spring and late leaf senescence in autumn in the ability of buckthorn to invade the understory of deciduous forests of North America. Light capture before canopy closure had a large impact on buckthorn growth and suggests that this early season light is important for the success of buckthorn in the forest understory.

Growth

Removing the high light windows in the spring and the fall resulted in a significant decrease in stem diameter growth, with spring shading having a more pronounced impact. Plants shaded in the spring had no stem diameter growth the first year and only a small increase the second year, while those shaded in the fall were only marginally impacted. Results of cohort two, which was not impacted by insect activity, confirmed the 2012 spring shading results from cohort one. The results of this study support the estimates by Harrington et al. (1989) and Fridley (2012), which calculated a higher annual carbon gain during the spring window than the fall. It makes sense that the removal of the spring window in this study resulted in more stressed plants, because sunlight is more

intense in the spring and plants are not in the process of going into the first stage of dormancy in preparation for winter.

Shading did not have a large effect on the height attained by the individual buckthorn plants, but this is not surprising as increased shade has been known to increase shoot elongation as the plant searches for light (Pickett and Kempf 1980). Woody plants tend to prioritize apical growth over lateral growth (Tolvanen 1995). One season shading was not enough to cause a decrease in height, as the spring shaded and both shaded groups were able to maintain normal height growth in 2012 and the fall shaded group was able to maintain normal height growth in 2013. It was not until two successive years of shading had been received that plants began to demonstrate decreased growth. The fall shaded group only received one round of treatment before measurement in 2013, but based upon the trend of decreased growth, it is likely that it too would have been significant after two years of removal. This delay suggests that there is a lag effect to shadings impact on height. While every effort was made to include plants with small root stores prior to the start of study, it may be that pre-study root stores allowed the plants to maintain normal height growth, and shading depleted these reserves for the next year.

Mortality

Despite declines in growth we did not observe changes in survival in this study. Only two individuals died over the two-year study. We do note that both were from groups that received spring shading. The shaded plants maintained

lateral and apical branch extension, but had very little stem diameter growth. As a decrease in stem diameter growth has been linked to increased mortality (Caspersen and Kobe 2001), I expect that over a longer study, mortality of buckthorn seedlings likely would have been observed.

Young plants were deliberately selected to avoid plants with large carbon stores as large carbon stores have been shown to be effective in helping plants overcome herbivore and light-induced stress (Myers and Kitajima 2007, Poorter and Kitajima 2007). It is unclear how much stored carbon each plant had at the start of the study, but I hypothesize that these reserves allowed the buckthorn to persist during the study. The decrease in stem diameter growth shows that these plants were running at decreased carbon capacity for the year, and likely had to make use of their reserves to survive. Even if the shaded plants were able to produce a net carbon gain for the year, the amount of carbon stored would have likely been small based on the decreased growth, leaving these buckthorn seedlings more susceptible to stress induced damage from herbivores or drought. It is likely that higher mortality rates would have been seen had the study continued.

Phenology

There was significant acceleration of leaf drop in the both shaded group in 2012 (Table 2) and trend towards early leaf fall in 2013 for the both shaded group in cohort one. Fall shading does not appear to be the mechanism driving this early abscission as the groups that only received shading in the fall retained their

leaves the longest. Shade has been shown to increase leaf longevity (Hidema et al. 1991, Reich et al. 1992, Hikosaka 2005, Brunel-Muguet et al. 2013), but does not explain why the both shaded group did not follow suit. However, these studies look at shading during the entire growing season not just additional shading in the fall. As all study groups received natural canopy shading for a minimum of 122 days in 2012 and 154 days in 2013 during the summer growing season additional shading may not have influenced leaf longevity via the same mechanisms in those studies. Instead, the loss of the spring light pulse combined with the loss of the fall light pulse and the deeply shaded summer would have left the both shaded treatment plants with a significant decrease in carbon reserves. Under these conditions the photosynthetic rewards of holding onto your leaves longer might not be worth the risk of damage from an early freeze. Triggering the plants to assimilate as much carbon and nutrients as possible from the leaves to ensure sufficient carbohydrate reserves to survive the winter.

In 2013, shading treatments in the spring delayed leaf expansion for both cohorts. Physiologically this makes sense as the rate of leaf expansion has been shown to decrease when plants have decreased sugar reserves (Pantin et al. 2011), and shading has been linked to slower leaf expansion (Granier and Tardieu 1999). Since these plants were light limited during expansion they may not have had the carbohydrates available to complete expansion at a rapid rate. It is surprising that this same trend was not seen in 2012 when spring shaded buckthorn had a similar deficit in light availability. If leaf expansion was delayed

by low photosynthetic rates in the spring, we would have expected that similar results would have been detected. It may be that there is a lag effect caused by decreased sugar reserves from the previous year, but since cohort two who did not experience these decrease in carbohydrate reserves in 2012 showed the delay in 2013 this explanation is unlikely. Another possible explanation for no delayed leaf expansion being detected in 2012 is the sensitivity of phenology sampling failed to detect it in year one. There is a large variation in the size of fully developed buckthorn leaves (Harrington et al. 1989) and determining when a leaf reaches full expansion can be difficult. In 2012 it was noted that the leaves on the young buckthorn study seedlings were considerably smaller than nearby adult plants (personal observation), and this led to a false perception that the leaves were still expanding. This delayed the recording of 50% leaf expansion and decreased the sensitivity of the observations in 2012. In 2013 having seen the full leaf out of plants, the observer was able to more accurately determine the timing of leaf expansion and was able to detect the differences in leaf expansion rates.

As shading caused a decrease in leaf expansion, early leaf out can have a positive feedback on the benefits of early bud break. Invasives, like buckthorn, that break bud under open canopy conditions will be able to get a full flush of leaves faster than later breaking natives. This would lead to not only a longer growing season, but also a more productive one as the high light environment

will stimulate earlier breaking species to reach full leaf expansion faster than late breaking species.

Insect damage

The observed insect damage was surprising as release from insect damage has been cited as one of the advantages that buckthorn exhibits in its North American range (Knight et al. 2007). Moreover, a study in 2005 found five groups of generalist insect families, including Lepidoptera tortricidae, feeding on buckthorn in Minnesota, but did not observe substantial damage (Van Veldhuizen et al. 2005). The high herbivore impacts in the current study could be the result of several processes: favorable microclimates for the caterpillars created by shade structures, exclusion of predators by the shade structures, or lower defense compounds in shaded leaves.

Caterpillars in the family Lepidoptera tortricidae are generally called leaf rolllers for their habit of tying leaves together to create a shelter to protect themselves from predators (Freeman 1958). The shade structures may have created a more favorable environment for the caterpillars by protecting them from predators and allowing larger populations to build up on the shaded buckthorn plants. The dense shade provided by the structures may have drawn the caterpillars to the plants, because the lower light levels mimicked denser foliage, which would naturally provide more cover from predation.

Changes in leaf chemistry between the shaded and unshaded plants may also have played a role in herbivory levels. Lepidoptera tortricidae caterpillars

prefer young shoots that contain less defense compounds (Aide and Londoño 1989) and larvae will disperse to higher quality browse to enhance feeding (Forkner et al. 2008). As lower levels of defense compounds have been linked to lower light availability (Waterman et al. 1984, Dudt and Shure 1994), it is possible that shading prevented the leaves from synthesizing the carbon needed to develop defense compounds. Making the shaded plants more susceptible to insect attack due to the increased palatability of the shaded leaves.

The possibility that these lepidopterans could serve as a biological control is exciting, but unlikely. Increased insect herbivory did not cause a significant decrease in plant growth and shading large portions of the forest is impractical. It is much cheaper to cut down buckthorn then put up shade cloth, and mechanical control is less likely to impact native vegetation.

Caveats

Light levels at these two stands averaged 2% to 3% transmittance during the summer growing season with a closed canopy. Shade treatments reduced light levels in the spring and fall to below 5% transmittance creating a light environment similar to that experienced by later breaking native plants and effectively removing the high light pulse in the spring and in the fall. However, during the spring and fall of 2012, light levels dipped below 1% for treated individuals. Plants were exposed to these low light levels for only a short period of time: 6 days for both sites in the spring and 12 days for Cedar Creek and 15 days for Warner Nature Center in the fall. These lower levels may have put

buckthorn below its light compensation point and exacerbated the effects of the treatments. The light compensation point for buckthorn could not be found in the literature, but buckthorn seedlings exhibit strong survival when grown in shade levels as low as 0.3% (Grubb et al. 1996). Light levels never approached this level, so it is probable that treatments did not result in buckthorn falling below its light compensation point. Furthermore, our more conservative light treatment in 2013 resulted in similar reductions in growth of shaded plants supporting the key role of seasonal light for buckthorn growth.

Conclusions

This study supports the hypothesis that early bud break and later senescence enhance buckthorn growth in the understory of North American forests. This strategy provides buckthorn windows of high light in spring and fall that support growth and survival despite low light conditions in summer. Thus, in North America, buckthorn is able to colonize more densely shaded forests than it does in its native range. This has several of implications for forest managers. As the period of high light in the spring is the most important for its survival, control measures should be enacted early in the spring to decrease its ability to take advantage of the high light levels. Secondly, non-native species that demonstrate early bud break compared to native canopies have a greater likelihood of becoming invasive and should be monitored as such.

Year	Treatment	Event	Warner Nature Center	Cedar Creek
2012	Spring shading	Start	3/16/12	3/21/12
		Stop	5/14/12	5/17/12
		Total days of shading	59	57
	Fall shading	Start	9/13/12	9/15/12
		Stop	11/27/12	11/27/12
		Total days of shading	75	73
2013	Spring shading	Start	5/1/13	4/30/13
		Stop	5/28/13	5/24/13
		Total days of shading	27	24
	Fall shading	Start	10/29/13	10/29/13
		Stop	11/13/13	11/14/13
		Total days of shading	15	16

Table 1: The timing of when spring and fall shading were applied to their respective treatment groups by site. During spring shading the spring shaded and both shaded groups were covered by shade cloth and during fall shading the fall shaded and both shaded groups received shading treatment.

Phenology	Treatment	2012 Day of the Year mean ± SE	2013 Day of the Year mean ± SE
Bud break	Ambient	77.9 ± 0.6	120.5 ± 0.1
	Spring shaded	78.9 ± 1.2	122.2 ± 1.3
	Fall shaded	79.9 ± 1.9	120.5 ± 0.1
	Both shaded	77.6 ± 0.5	121.6 ± 1.2
Expansion	Ambient	126.3 ± 0.3	143.6 ± 1.0
	Spring shaded	126.6 ± 0.6	150.1 ± 1.4 *
	Fall shaded	129.3 ± 1.2 *	144.8 ± 1.1
	Both shaded	127.2 ± 0.6	151.0 ± 1.5 **
Senescence	Ambient	290.6 ± 1.7	312.2 ± 1.3
	Spring shaded	293.4 ± 1.3	308.9 ± 2.9
	Fall shaded	294.2 ± 1.3	311.4 ± 2.0
	Both shaded	289.0 ± 1.6	303.6 ± 4.7
Leaf Drop	Ambient	295.1 ± 0.9	312.7 ± 1.4
	Spring shaded	295.1 ± 1.0	309.9 ± 2.9
	Fall shaded	297.7 ± 1.3	312.8 ± 2.2
	Both shaded	289.7 ± 1.6 ***	304.0 ± 4.7

Table 2: Summary of mean phenology parameters for each treatment by the two study sites for Cohort 1. Dates are given by day of the year with Jan 1 equaling day 1. Asterisks denote significant differences between the ambient and marked groups (* < 0.05, ** < 0.01, *** < 0.001).

Phenology	Treatment	Day of the year Mean ± SE
Bud break	Ambient	120.4 ± 0.1
	Spring shaded	120.5 ± 0.1
Expansion	Ambient	147.4 ± 0.9
	Spring shaded	151.7 ± 1.0 *
Senescence	Ambient	310.2 ± 2.4
	Spring shaded	306.1 ± 2.5
Leaf Drop	Ambient	311.1 ± 2.5
	Spring shaded	306.6 ± 2.5

Table 3. Cohort two mean phenology response data for the 2013 growing season. Means are and average for all plants at both sites. Dates are given on the basis that Jan 1 = 1, and asterisks denote a $p \leq 0.01$ between treatments.

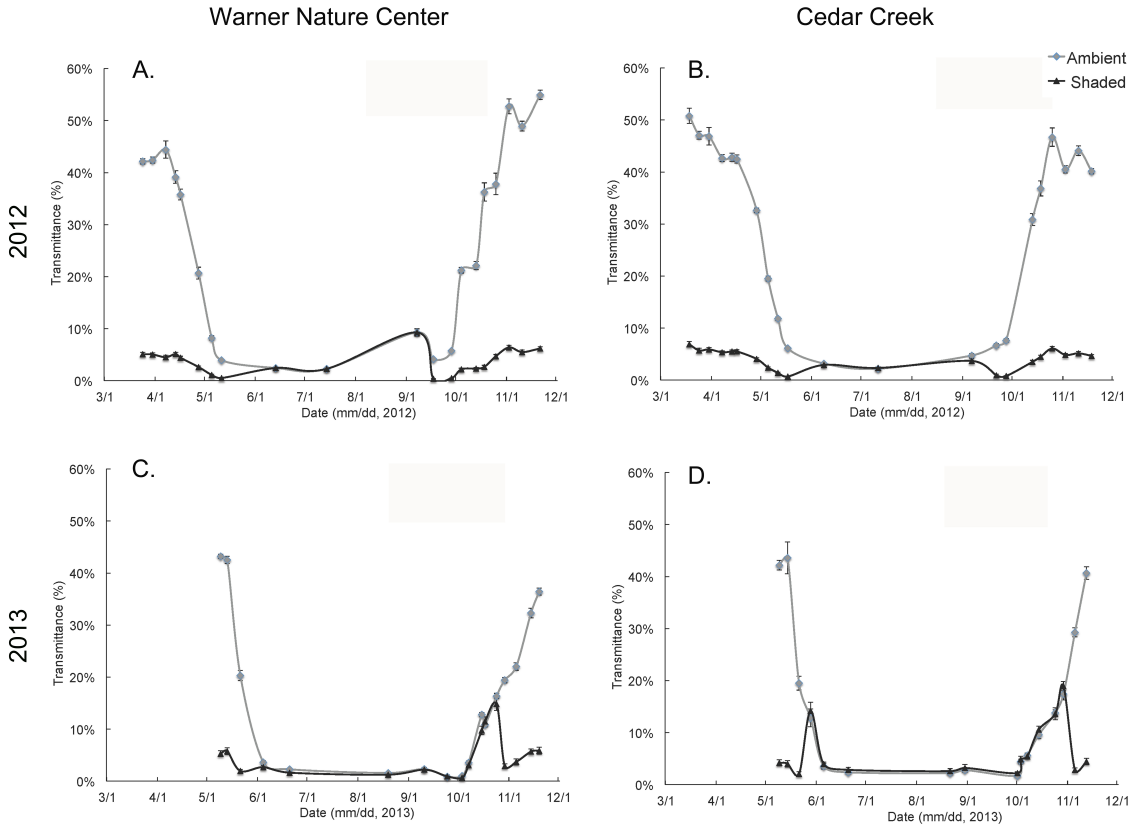

Figure 1. Percentage of solar radiation reaching ambient and shaded study plants during the growing season in 2012 for Warner Nature Center (A) and Cedar Creek (B), and in 2013 for Warner Nature Center (C) and Cedar Creek (D). In 2012 shading was applied when percent transmittance was above 5% in the spring and the fall. During 2013 shading was applied when percent transmittance was above 20%. During the summer growing season no treatment was applied.

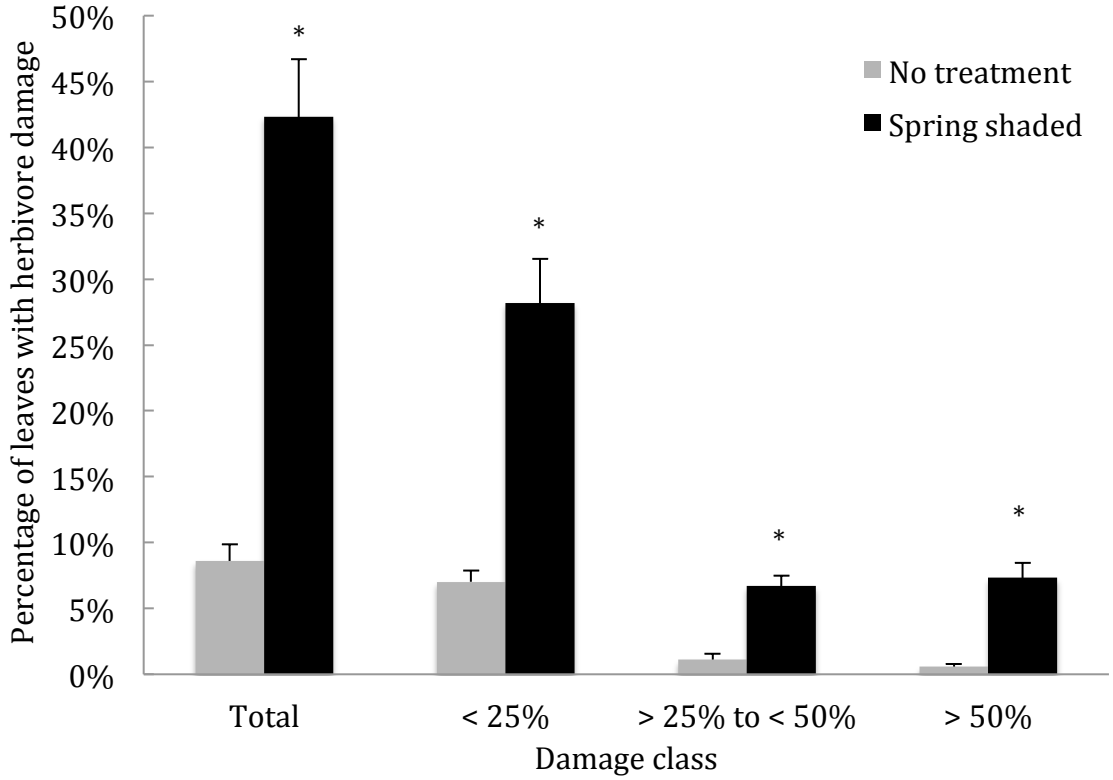

Figure 2. Comparison of the percent of leaves with varying levels of insect damage between plants that received spring shading (spring shaded and both shaded) and plants that received no treatment (ambient and fall shading) for cohort one on May 22, 2012 at both sites. Plants in the spring shaded treatment had significantly higher levels of herbivory. Error bars display standard error. * = $p < 0.0001$

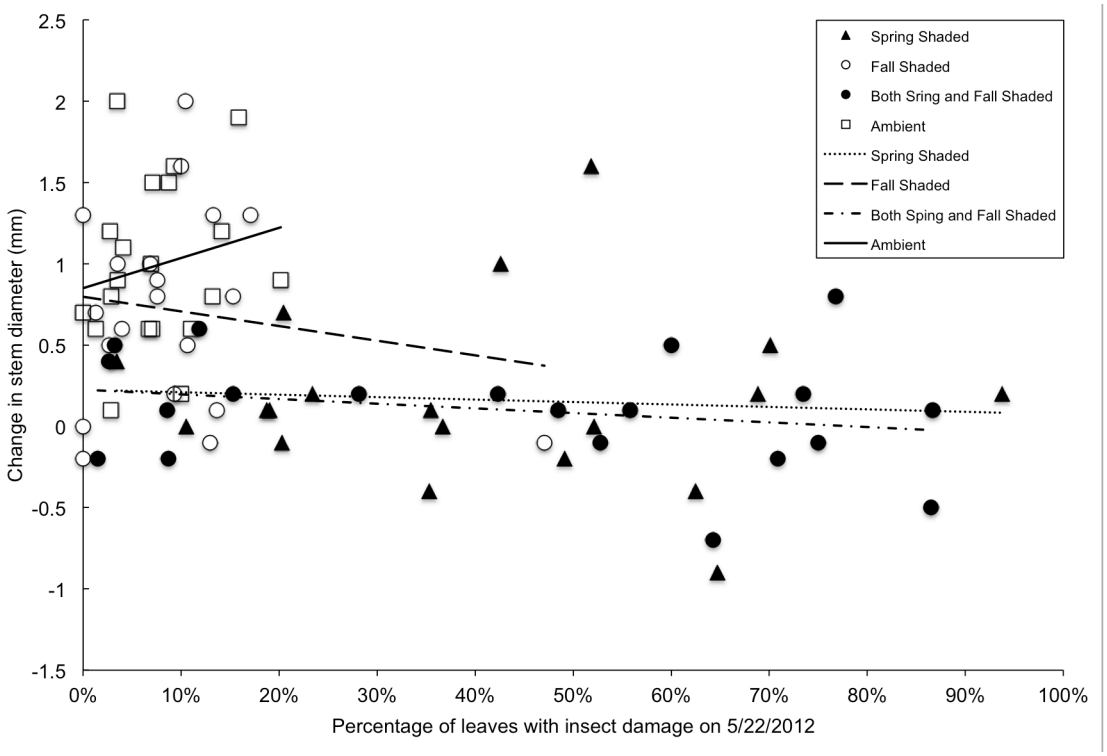

Figure 3. The relationship between percentage of leaves with insect damage on May 25, 2012 and change in stem diameter over the growing season (March 2012 to September 2012) for seedlings of common buckthorn (*Rhamnus cathartica*) growing at two sites in central Minnesota and subjected to four shade treatments. Increased herbivore damage was not correlated with a decrease in stem diameter change.

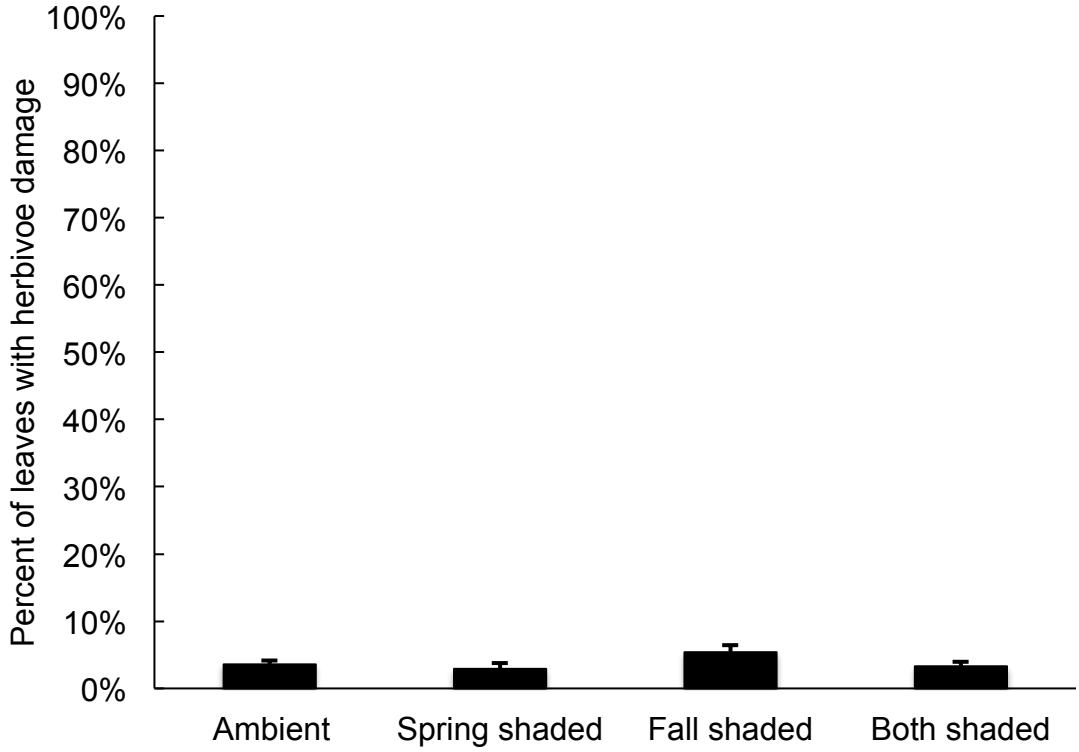

Figure 4: The total number of leaves with Insect damage from survey performed on June 9, 2013 for cohort one at both sites. Treatment was not a significant predictor of insect damage ($p > 0.05$).

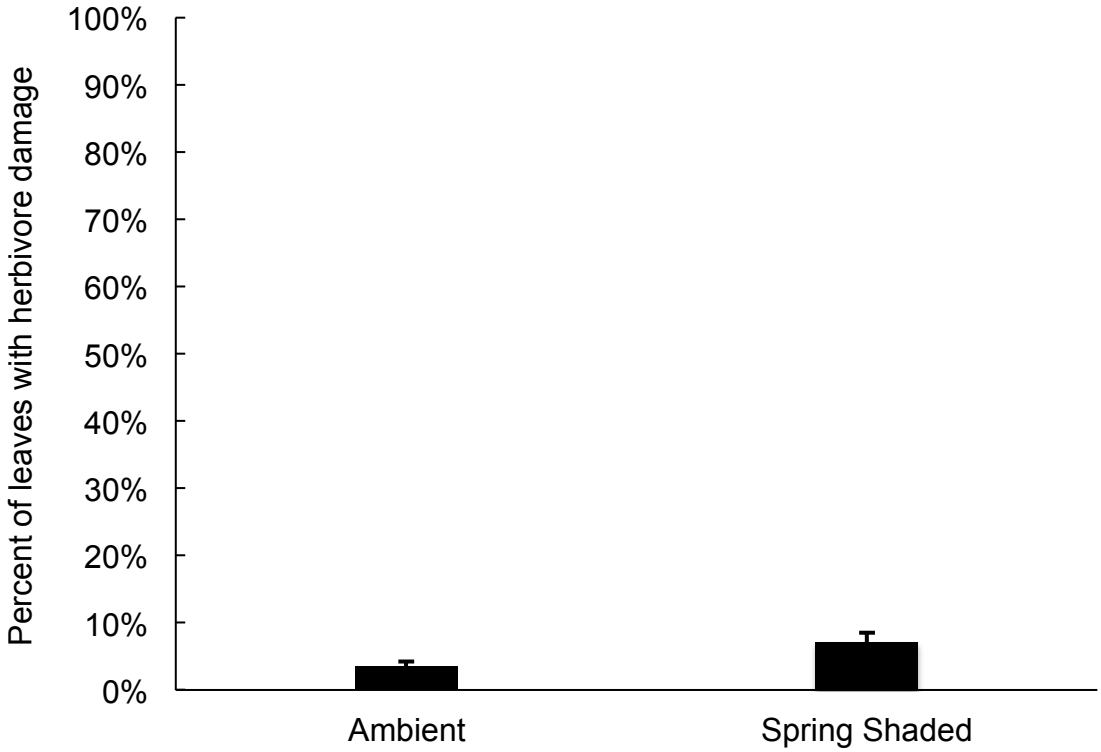

Figure 5: Comparison of herbivore damage in cohort 2 on June 9, 2013, showing that herbivore damage was low and not associated with treatment ($p > 0.05$).

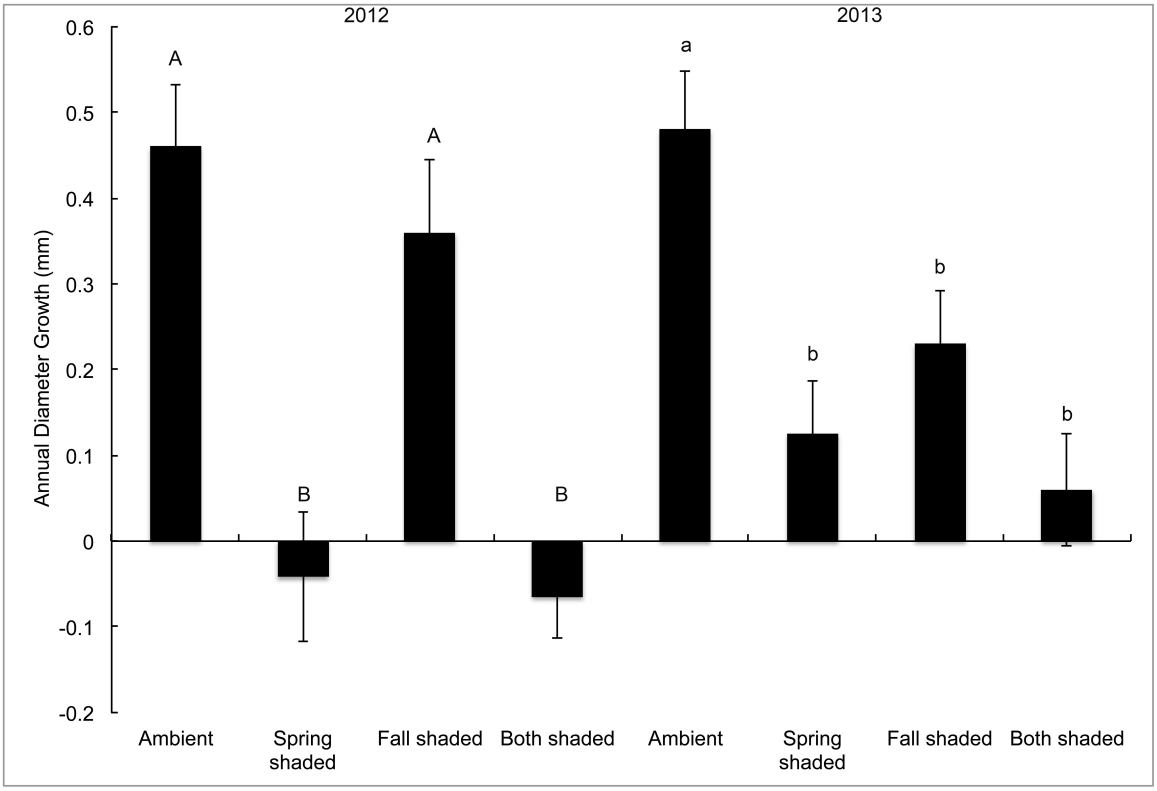

Figure 6. Change in stem diameter (+ 1 SE) over two growing seasons by treatment groups is shown for the periods of March 16, 2012 to September 1, 2012 in 2012 and May 1, 2013 to September 1, 2013 for 2013. On September 1, 2012 the ambient and fall shaded had significantly larger growth compared to the spring shaded and both shaded treatments (* = ANCOVA($F_{3,75}$ = 15.2, P < 0.0001)). By September 1, 2013 the ambient group had significantly more stem growth than all three treatment groups (. ** = ANCOVA($F_{3,75}$ = 8.9, P < 0.0001)). Differences between groups for each year calculated by ad hoc Tukey HSD test and denoted by different letters.

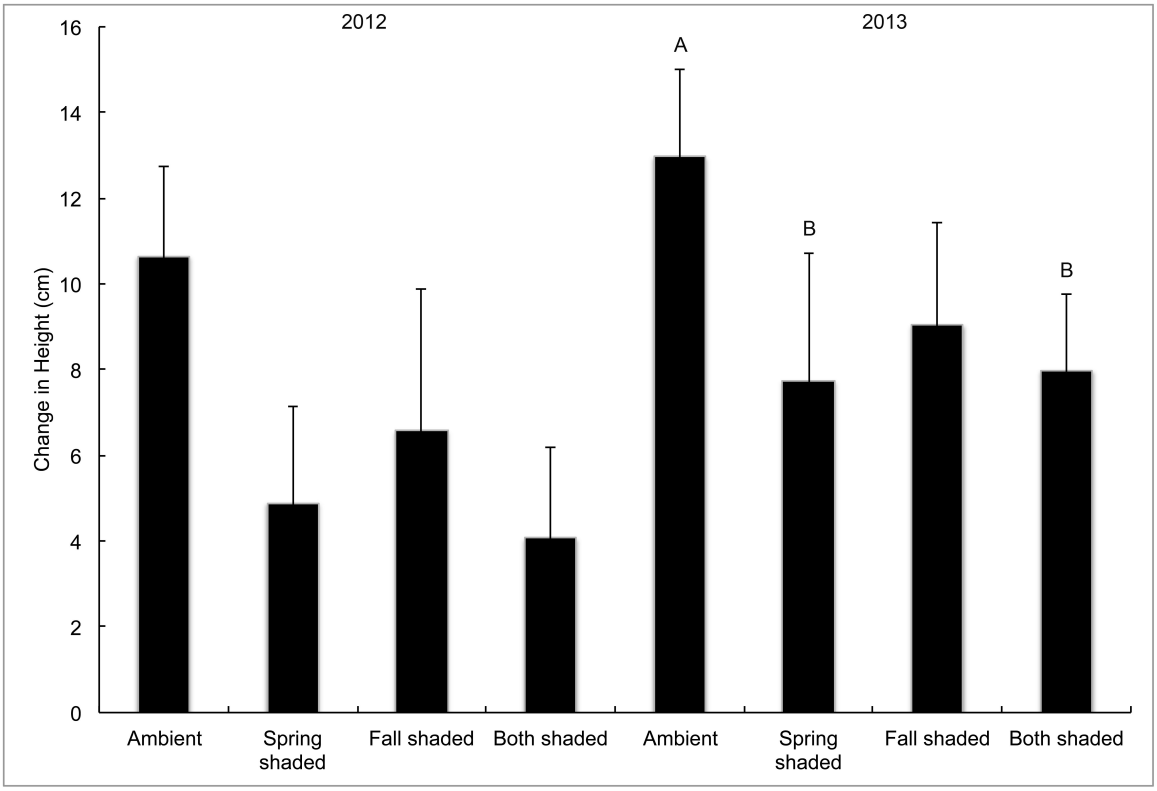

Figure 7. How the average height for each group changed for cohort one from their initial measurement on March 16, 2012 over two growing seasons by treatment. Height was not significant in 2012 but in 2013 the ambient group was significantly taller than both the spring shaded and both shaded groups ($F_{3,76} = 3.74$, $p = 0.01$). Significance between groups analyzed via Tukey HSD test and denoted by different letters.

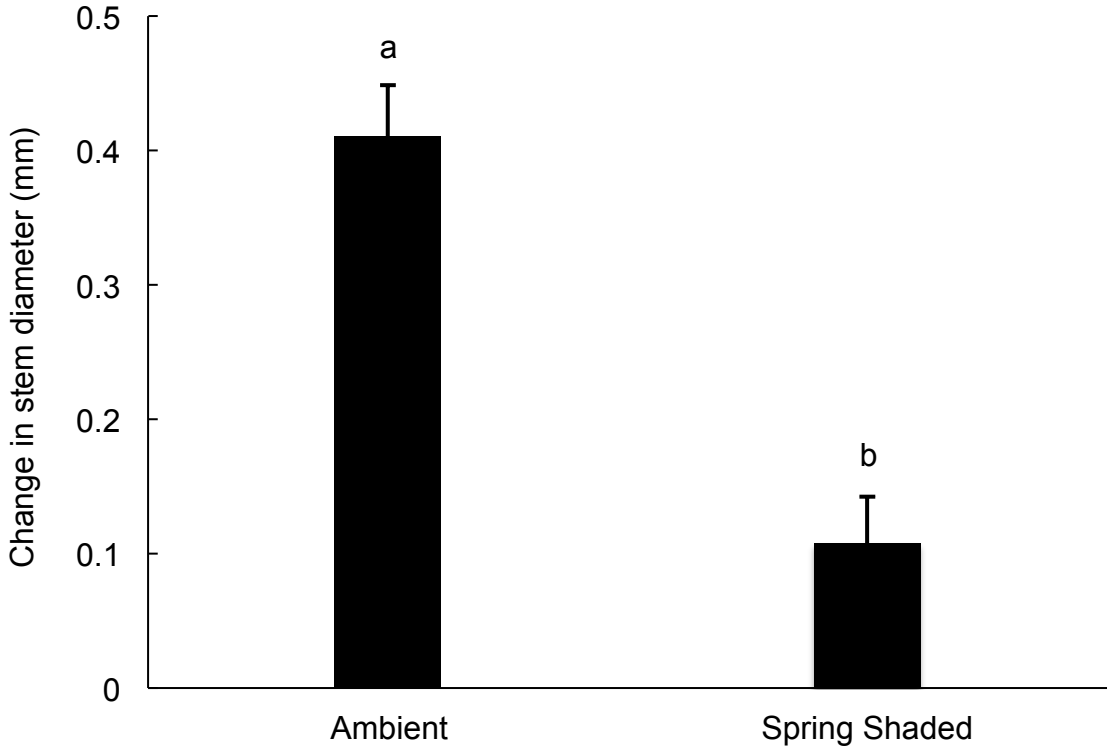

Figure 8. Change in stem diameter for cohort two from May 2013 to September 2013. Treatment was a significant predictor of change in stem diameter for cohort 2 in 2013 ($F_{3,75} = 11.5$, $p < 0.0001$). Different letters denote significant differences.

Chapter 2: The effects of winter chilling on timing of budburst in *Rhamnus cathartica*

Introduction

Over a thirty year period from 1983 to 2003 surface temperatures increased by an average of 0.2^{o}C per decade (Hansen et al. 2006), and during the same period European plant communities showed an 8 day increase in growing season length due to early leaf out and later scenscence (Chmielewski and Rötzer 2001). In general, global warming is causing spring phenology to advance in many plant and animal species (Aono and Kazui 2008). Warm temperatures have caused spring events to increase by 2.5 days per decade on average since 1971 (Menzel et al. 2006). Climate change models predict that the Earth will continue to warm by 2 to 4 degrees this centurey and that the timing of spring phenology events will be impacted by these warmer climate patterns (IPCC 2014).

Changing phenology with climate change could have both positive and negative impacts. Plant and animals use different cues to determine when to emerge from dormancy and changing phenological patterns can cause a mismatch in the timing of plant-animal interactions (Hegland et al. 2009). Plant response to climate change varies among species and differing rates of spring advancement under climate change could change the dynamics of resource competition between species. Species that break bud earlier have longer growing seasons, and could have a competitive advantage over plants that are slower to respond to climate change (Harrington et al. 1989). However, timing of spring leaf out represents a balancing act between extending the growing season while

minimizing frost risk. Climate models predict that early spring phenology will expose plants to more harmful late season frosts that can damage tender growth (Hänninen 1991, Rigby and Porporato 2008). To understand and predict changing phenology with climate change, we must understand the mechanisms that control dormancy break in plants.

For perennial plants there are three main factors that influence the timing of dormancy break in plants: forcing temperatures in the spring, day length, and winter chilling (Körner and Basler 2010). The importance of these three factors varies in plants and depends upon the life history strategies and climate in which each species evolved (Körner and Basler 2010). Thus, not all species respond the same to climate change. Each species must be tested individually, to determine the relative importance of chilling, forcing and photoperiod, making it difficult to create general models of plant phenological responses.

In many plants, the earlier onset of warm temperatures in spring may not always result in earlier spring leaf out. Warm temperatures alone are not enough to cause bud break in many cold weather adapted plants, as an early bud break followed by a frost could be damaging to a plant. Many plants rely on winter chilling and day length to confirm that it is safe to break bud. Once individual thresholds for these factors have been met plants will respond quickly to warm spring temperatures, but if these have not been met plants require greater quantities of spring forcing temperatures and bud burst timing is delayed (Heide 2003). As climate change will cause both a decrease in winter chilling with

warmer winters, and increasing spring temperatures, to model plant interactions we must understand how these opposing forces act upon the timing of bud burst for each species. Insufficient winter chilling could become a problem for many species under the predicted climate change forecasts. There has been a strong push in the scientific community to determine native species response to winter chilling requirements (Laube et al. 2013)(Orlandi et al. 2002, Kaduk and Los 2011, Polgar et al. 2014), but few have looked at invasive species.

Invasive species present an interesting case for studying the effects of climate change on phenology. Many of these species evolved in different climates than the environments they are invading, and their response may be different than those of native species. Many invasive species impacting our North American ecosystems evolved in warmer, Asian and European climates, and climate change is causing their invaded range to more closely resemble their native range. These species may be predisposed to the new climatic regimes present under global warming (Hellmann et al. 2008). To understand how these species may react we must understand how the drivers of spring bud break affect them.

Rhamnus cathartica (buckthorn) is native to the more moderate climate of Europe and Asia, but has become invasive in North America (Knight et al. 2007). Buckthorn tends to be the first species to break bud in the spring (Knight et al. 2007), and it is thought that this phenology response could provide it a competitive advantage over native species (Harrington et al. 1989). Here, I

present data on the sensitivity of buckthorn budburst to mid-winter chilling. Since buckthorn tends to break bud early in the season, we hypothesize that buckthorns will have low sensitivity to mid-winter chilling.

Methods

In December 2012, ten *Rhamnus cathartica* shrubs, 2 to 3 m in height, were identified at Cedar Creek Ecosystem Science Reserve in East Bethel, MN (45°25'N, 93°10'W), with a mean annual temperature of 6.7°C and mean precipitation of 72.6 cm/year. Starting December 19th 2012 and continuing every two weeks until March 28, 2013 when temperatures averaged above 0°C, one 30 cm branch clipping from each shrub was collected for temperature forcing. Trimmed branches were stored on ice during collection and transportation to a greenhouse at the University of Minnesota St. Paul, MN campus for processing. To expose fresh vascular tissue, the bottom 5 mm of each branch was removed and the base of the branch was immediately submerged in water. The branches were trimmed and the water was changed weekly, to ensure proper water flow through the vascular tissue. Branches were forced in a greenhouse maintained at 20°C, with a 14 hour photoperiod, from time of collection till bud break. Branches failing to break bud after 90 days were marked as dead and discarded.

I observed phenology three times per week and buds were scored in two phenophases: bud swelling and bud break. Buds were considered swollen when the scales began to swell exposing green leaf tissue below the surface of the bud, and plants were in bud break when the scales had burst open and leaf

tissue was visible extending through the tip of the bud (Figure 1). Once one bud reached the developmental stage, the entire twig was classified as reaching that developmental time point.

We used weather data from a field monitoring station at Cedar Creek Ecosystem Science Reserve located less than 1 km away from the twig collection site. Winter chill days were defined as days with an average temperature reading at or below a base temperature of 5^{o}C. The sum of the daily chill days from November 1, 2012 to date of branch collection was the total winter chill days each branch received. The baseline of 5^{o}C was chosen for cooling as it is the most commonly cited temperature in the literature, and there was no difference when results were analyzed with 10^{o}C, 7^{o}C, and 0^{o}C base temperatures. Forcing days were calculated as the total number of days in the 20^{o}C greenhouse until budbreak.

I used analysis of variance to analyze the sensitivity of budburst to winter chilling. The model used the fixed effect of the number of chill days prior to collection with a response variable of the number of days until bud burst to test sensitivity in winter chilling. To test differences between groups a separate model with collection number as a fixed effect and days until bud burst was analyzed. Tukey HSD test was run to test differences between individual collections.

Results

Collected branches were responsive to the greenhouse forcing temperatures indicating that the chill requirements for breaking dormancy were met even by mid-December. Of the 80 branches sampled in this study, only one branch failed to break bud. Since there was no way to determine if the branch died prior to collection, was mishandled during the forcing, or lacked sufficient winter chilling for dormancy release, this branch was removed from the analysis. For the final analysis seven collections with n=10 branches and 1 collection with n=9 (January 7, 2013) were analyzed.

The number of chill days was a significant predictor of days to bud break after transfer to forcing conditions ($F_{1,77} = 335.7$, $p < 0.0001$, Figure 2). Number of days to break bud decreased linearly from 30.7 days for cuttings harvested at the onset of the study, to 10.9 days at collection 7 (March 14, 2013), after which point no further drop was recorded.

To analyze differences among collection dates a model was run with collection date as the predictor variable and time to bud break as the response variable. Collection number was a significant predictor of bud break ($F_{7,71} = 52.5$, $p < 0.001$). Tukey HSD analysis of bud break by collection indicated significant differences between the first 6 collections, and no difference between the 6th through 8th collection, indicating that on the time of the 6th collection (February 28, 2013) the plants had reached their maximum level of chilling. Chilling beyond this was not responsible for a change in the time to bud break upon exposure to forcing temperatures.

Discussion

Winter chilling influences the timing of spring phenology events, and understanding how it impacts individual plants will allow researchers to better model when plants will break bud under future climate change scenarios. This study demonstrates that winter chilling is important for regulating the timing of budburst in buckthorn in the spring. Accumulating winter chill days below 5^{o}C resulted in accelerated spring bud break up to 137 chill days. Additional chilling after this time point caused no appreciable response in budburst. Plants that do not receive this level of chilling, will take longer to break bud after the onset of warmer weather. Thus, predicting buckthorn budburst times in a warmer climate will need to take into account winter chilling.

Lack of winter chilling did appear to lead to failure to break bud. In similar studies with other species, a higher percentage of branches often failed to break bud at lower chilling levels (Laube et al. 2013). In most species, 10 to 20 percent failed to break bud, but in the case *Fagus sylvaticus* and *Quercus rubra* less than 33 chill days resulted in 90 and 80 percent failures respectively, and under longer chilling regimes 100 percent of both species successfully broke bud (Laube et al. 2013). Since only one buckthorn branch out of 80 failed to break bud in this study, I conclude that all branches in this study exceeded the minimal level of

cooling needed to break dormancy. This suggests that buckthorn has a low minimal chilling threshold for dormancy release.

This low winter chilling requirement could be a potential boon for buckthorn in North American forests. Even under the most extreme climate change predictions Minnesota is unlikely to see winters warm enough to provide less than 48 chill days. Chances of buckthorn failing to break bud due to insufficient winter chilling are very small. Conversely many native tree species with which buckthorn competes have higher requirements for dormancy release and thus could suffer long delays or complete failures to break bud in a warmer climate (Laube et al. 2013, Montgomery unpublished data). Buckthorn would not be as strongly affected, positioning it to take advantage of warmer springs and thus providing it with greater access to light and other resources.

Buckthorn's maximum winter chilling threshold could cause the opposite effect at lower latitudes. Spring forcing temperatures present before buckthorn received 119 days of winter chilling resulted in slower bud break. At lower latitudes when competing against species with lower maximum chilling thresholds, the delay experienced by buckthorn by not receiving its maximum chilling could result in a competitive disadvantage if other plants are able to break bud sooner.

There are a couple of caveats with this study. While each treatment group received different amounts of chilling, collections were not all done at the same time. Branches that received less chilling were brought into the greenhouse

earlier than those with more chilling. The time since leaf fall was not consistent and there was no way of isolating this factor with the current study design. Correspondingly, in later collections the day-night cycle used in the greenhouse more closely matched the cycle the plants were experiencing in the field. All branches in the greenhouse received the same 14 hour day cycle, and the more drastic difference between natural and greenhouse circadian rhythms could have affected the timing of bud break in early collections. To eliminate these variables, work in controlled growth chambers where plants receive different levels of chilling over the same time period should be done.

It is clear that winter chilling plays a role in the timing of spring bud break for buckthorn. Plants that receive more chill days are faster to respond in the spring, but low levels of chilling did not cause a major problem for buckthorn. The slower response by buckthorn to spring temperatures was still relatively fast when compared to many North American species and a minimum winter chill threshold that resulted in bud break failures or longer delays was not detected by this study. Buckthorn's ability to break bud quickly after spring warm up compared to many native species should position it to do well under future climatic regimes.

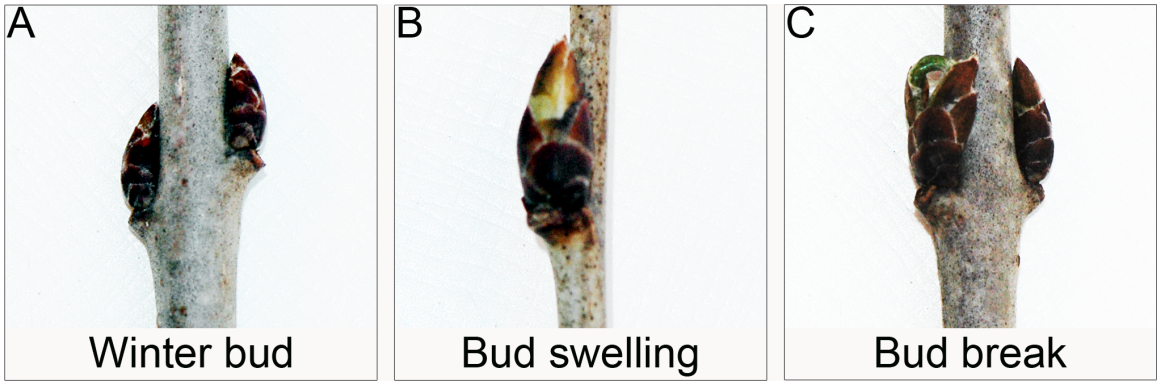

Figure 1. Examples of the three phenology stages of buckthorn. Panel A shows a normal winter bud at time of collection. Panel B shows a bud undergoing bud swelling, with the scales beginning to pull apart and an area of yellow seen through the scales, as the bud prepares to break. In panel C the scales have broken open and the tips of new leaves are visible. Note that images are not to scale and the increase in size between the buds is exaggerated.

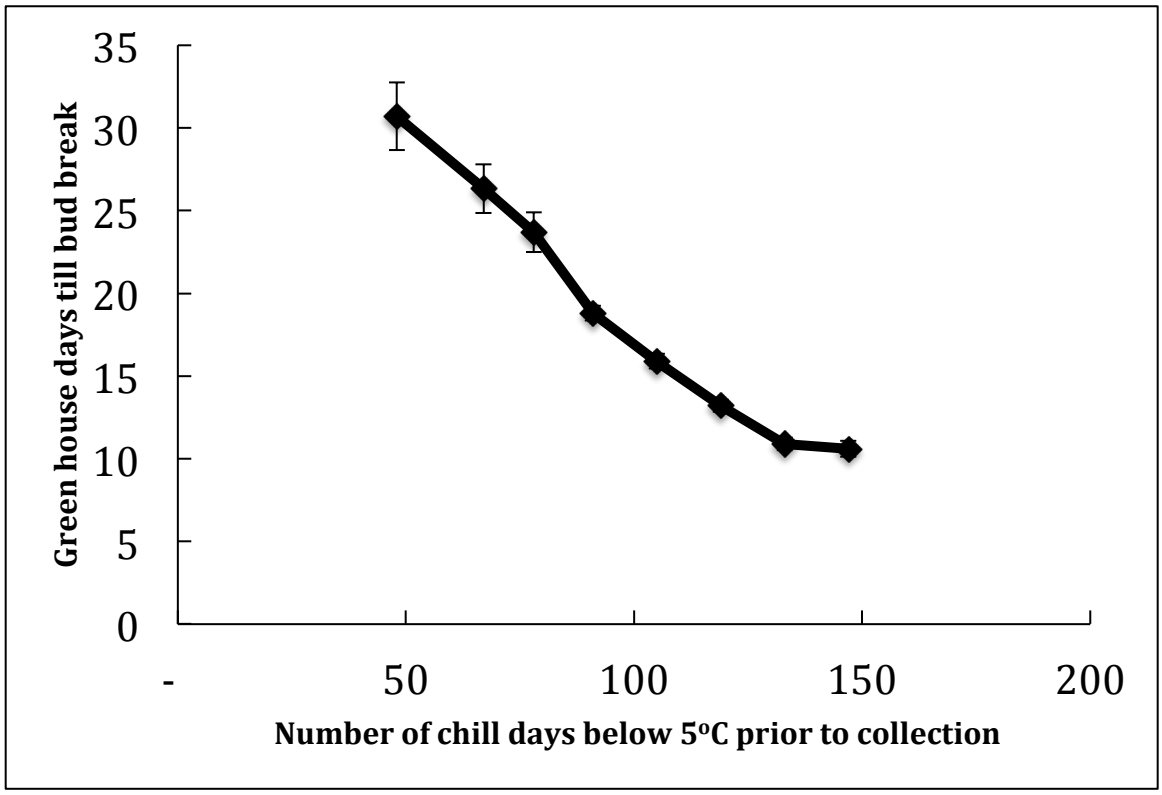

Figure 2. The relationship between number of chill days and the number of days of forcing at 20°C required for budburst in twigs of *Rhamnus cathartica* (common buckthorn collected from Cedar Creek Ecosystem Science Reserve. Error bars are standard error.

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